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(54) Title: USE OF A DOUBLE-STRANDED RIBONUCLEIC ACID FOR SPECIFICALLY INHIBITING THE EXPRESSION OF A GIVEN TARGET GENE

(57) Àbstract: [in English]

Use of a double-stranded ribonucleic acid for specifically inhibiting the expression of a given target gene.

The invention concerns the use of a double-stranded ribonucleic acid (dsRNA) for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell. Furthermore, it concerns the use of such a ribonucleic acid to produce a medicament, the medicament, and a method for specifically inhibiting the expression of said target gene in a cell.

A method for inhibiting the expression of a target gene in a cell is known from DE 101 00 586 B1, wherein an oligoribonucleotide with double-stranded structure is introduced into the cell. One strand of the double-stranded structure is complementary to the target gene.

It is known from Elbashir, S. M., et al., Nature 411 (2001), pages 494-498, that a short dsRNA in which three nucleotides are not complementary to the target gene hardly inhibits the expression of a target gene at all. A fully complementary dsRNA, on the other hand, produces an effective inhibition of the expression of the target gene.

It is known from Holen, T., et al., Nucleic Acids Research 30 (2002), pages 1757-1766, that an inhibiting of the expression of a gene by short dsRNA through RNA interference is also possible with dsRNAs whose one strand has one or two nucleotides not complementary to the target gene.

Many illnesses and degenerative conditions of cells are due to the fact that a gene important to cells, often a proto-oncogene, is altered by one or a couple of point mutations. The problem in the treatment of such an illness or such cells with the methods known thus far is that the inhibition of the expression of the mutated gene often leads to an inhibition of the nonmutated gene as well. This often results in serious side effects.

The problem of the present invention is to avoid the drawbacks of the prior art. In particular, it should provide for a use of a dsRNA for the specific inhibition of the expression of a target gene having a point mutation with regard to an original gene in a cell so that the expression of the original gene remains largely unaffected. Furthermore, it should provide a medicament and a method for the specific inhibition of the expression of a given target gene, as well as a use for the making of a medicament. This problem is solved by the features of claims 1, 2, 18 and 33. Advantageous embodiments will appear from the features of claims 3 to 17, 19 to 32 and 34 to 44.

According to the invention, there is provided a use of a double-stranded ribonucleic acid (dsRNA) for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein one strand S1 of the dsRNA has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene. The invention moreover concerns the use of such a dsRNA to make a medicament for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell. A nucleotide in the sense of this invention is "complementary" to the target gene or original gene if it can form a specific Watson-Crick base pairing with a nucleotide corresponding to it in its sequence position. By "complementary region" is meant that the nucleotides contained therein are basically complementary to the target gene. This means that not all nucleotides in the region are complementary to the target gene. The number of nucleotides which are not complementary to the target gene is one in the lowest case. By target gene is generally meant the DNA strand of the double-stranded DNA present in the cell, which is complementary to a DNA strand serving as the matrix during the transcription, including all transcribed regions. Thus, it generally involves the sense strand. The strand S1 can therefore be complementary to a RNA transcript formed during the expression or its processing product, such as a mRNA. For example, it can be sufficient for the strand S1 to be complementary to a portion of the 3'-untranslated region of the mRNA. But the target gene can also involve a portion of a viral genome. The viral genome can also be the genome of a (+)-strand RNA virus, especially a hepatitis C virus.

The original gene can be any given gene that differs from the target gene being inhibited merely in having one or a few point mutations. In general, it involves a wild type gene. A dsRNA will be produced if the ribonucleic acid consisting of one or two nucleic acid strands has a double-stranded structure. Not all nucleotides of the dsRNA need to have canonical Watson-Crick base pairing within the dsRNA. The maximum possible number of base pairs is the number of nucleotides in the shortest strand contained in the dsRNA. The region complementary to the target gene can have fewer than 25 consecutive nucleotides, in particular 19 to 23, preferably 20 to 24, especially preferably 21 to 23, in particular 22 or 23. The strand S1 can have fewer than 30, preferably fewer than 25, especially preferably 21 to 24, in particular 23 nucleotides. It has been found that short dsRNAs are especially efficient at inhibiting the expression of a target gene. These dsRNAs are also known as siRNAs (short interfering RNAs).

During the specific inhibition, the expression of the original gene is less inhibited than that of the target gene. In the ideal case, the expression of the original gene remains largely unaffected. For this, one specifically uses a dsRNA not optimally inhibiting the expression of the target gene. Thus, one can prepare a dsRNA which is so little complementary to the original gene that its expression remains largely unaffected. Side effects due to inhibiting the original gene can be avoided or mitigated.

The inhibiting of the expression by the dsRNA occurs preferably by the principle of RNA interference. The nucleotide not complementary to the target gene is preferably situated not at the 3' or 5' end of the region. Ideally, the noncomplementary nucleotide lies in the middle part of the region. The target gene can have one or two point mutations with respect to the original gene. In that case, the use for specific inhibiting of the expression of the target gene according to the invention is especially suitable for specifically inhibiting only this expression, but not that of the original gene.

In one embodiment of the invention, the original gene is a proto-oncogene and the target gene an oncogene derived from it. An oncogene often differs from the cellular proto-oncogene corresponding to it by only a single point mutation. The inhibiting of the expression of the oncogene with traditional dsRNA therefore usually also brings about an inhibiting of the expression of the corresponding proto-oncogene. This often entails so serious side effects that a use of traditional dsRNA for inhibiting the target gene in an organism is scarcely possible.

The cell can be a tumor cell. In one embodiment of the invention, one nucleotide of the region is not complementary to the target gene and two nucleotides of the region are not complementary to the original gene. Such a slight difference in the number of complementary nucleotides is already enough to almost totally inhibit the expression of the target gene and leave the expression of the original gene largely unaffected.

In one embodiment of the method, at least one base pair is not complementary within the dsRNA, i.e., the nucleotides of the base pair are not specifically paired according to Watson-Crick. By varying the number of noncomplementary base pairs within the dsRNA, the effectiveness of the dsRNA can be modulated. A reduced complementarity within the dsRNA lessens its stability in the cell.

Preferably, the dsRNA has a single-stranded projection formed from 1 to 4, especially 2 or 3 nucleotides at least at one end of the dsRNA. One end is a region of the dsRNA in which one 5' and one 3' strand end are present. Thus, a dsRNA formed only from the strand S1 has a loop structure and only one end. A dsRNA formed from the strand S1 and a strand S2 has two ends. One end is then formed by one strand end lying on the strand S1 and one lying on the strand S2. Single-stranded projections lessen the stability of the dsRNA in blood, serum and cells and at the same time strengthen the expression-inhibiting action of the dsRNA. It is especially advantageous when the dsRNA has the projection only at one end, especially its end having the 3' end of the strand S1. The other end is then formed smooth in the case of a dsRNA having two ends, i.e., without projections. Surprisingly, it has been found that one projection at one end of the dsRNA is enough to strength the expression-inhibiting action of the dsRNA, without lowering the stability to the same extent as by two projections. A dsRNA with only one projection has proven to be sufficiently durable and especially effective both in various cell culture media and in blood, serum and cells. The inhibiting of the expression is especially effective when the projection is located at the 3' end of the strand S1.

Preferably, the dsRNA has, besides the strand S1, also a strand S2, i.e., it is formed from two single strands. The dsRNA is especially effective when the strand S1 (antisense strand) has a length of 23 nucleotides, the strand S2 a length of 21 nucleotides, and the 3' end of strand S1 a single-stranded projection formed from two nucleotides. The end of the dsRNA located at the 5' end of the strand S1 is formed smooth in this case.

The strand S1 can be complementary to the primary or processed RNA transcript of the target gene. The dsRNA can be present in a preparation that is suitable for inhalation, oral ingestion, infusion and injection, especially for intravenous, intraperitoneal or intratumoral infusion or injection. The preparation can consist, and especially consists solely of a physiologically tolerated solvent, preferably a physiological salt solution or a physiologically tolerated buffer, and the dsRNA. The physiologically tolerated buffer can be a phosphate-buffered salt solution. It has been discovered, surprisingly, that a dsRNA administered and solved simply in such a solvent or such a buffer is taken up by the cell and inhibits the expression of the target gene, without the dsRNA needing to be packaged in a special vehicle for this.

Preferably, the dsRNA is present in a physiologically tolerated solution, especially a physiologically tolerated buffer or a physiological salt solution, or enveloped by a micelle structure, preferably a liposome, a virus capsid, a capsoid or a polymer nanocapsule or microcapsule, or bound to a polymer nanocapsule or microcapsule. The physiologically tolerated buffer can be a phosphate-buffered salt solution. A micelle structure, a virus capsid, a capsoid or a polymer nanocapsule or microcapsule can facilitate the uptake of the dsRNA into infected cells. The polymer nanocapsule or microcapsule consists of at least one biodegradable polymer, e.g., polybutylcyanoacrylate. The polymer nanocapsule or microcapsule can transport and release in the body the dsRNA contained therein or bound thereto. The dsRNA can be administered orally, by means of inhalation, infusion or injection, especially intravenous, intraperitoneal or intratumoral infusion or injection. Preferably, the dsRNA is used in a dosage of not more than 5 mg, especially not more than 2.5 mg, preferably not more than 200 µg, especially preferably not more than 100 µg, preferably not more than 50 µg, especially not more than 25 µg, per kg of body weight and per day. Namely, it has been found that the dsRNA has an excellent effectiveness in inhibiting the expression of the given target gene already in this dosage.

The invention furthermore concerns a medicament for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein the medicament contains a double-stranded ribonucleic acid (dsRNA), whose one strand S1 has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene. Preferably, the medicament is present in at least one administration unit which contains the dsRNA in an amount that enables a dosage of not more than 5 mg, especially not more than 2.5 mg, preferably not more than 200 µg, especially preferably not more than 100 µg, preferably not more than 50 µg, especially not more than 25 µg, per kg of body weight and per day. The administration unit can be designed for a onetime administration or ingestion per day. In that case, the entire daily dose is contained in one administration unit. If the administration unit is designed for an administration or ingestion several times during the day, the dsRNA is contained in a correspondingly smaller amount, making it possible to achieve the daily dose. The administration unit can also be designed for a single administration or ingestion over several days, by releasing the dsRNA over the course of several days, for example. The administration unit will then contain a corresponding multiple of the daily dose.

According to the invention, furthermore, a method is provided for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein a double-stranded ribonucleic acid (dsRNA) is introduced into the cell and one strand S1 of the dsRNA has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene.

For further advantageous embodiments of the invented medicament and the invented method, refer to the preceding embodiments.

The invention shall now be explained as an example by means of the drawings. These show:

Fig. 1, a graphical representation of the inhibiting of the expression of a HCV-luciferase fusion protein by dsRNAs, which are complementary to a sequence of a target gene in differing degree, and

Fig. 2, a graphical representation of the inhibiting of the expression of a HCV-luciferase fusion protein by dsRNAs, which are complementary to a sequence of a target gene in differing degree and which are partly formed from RNA strands not completely complementary to each other.

In order to make a reporter system, a sequence 26 nucleotides in length of a cDNA sequence, serving as the target gene and corresponding to the 3'-nontranslated region of a HCV-RNA, was fused with the open reading frame of the luciferase gene from the expression vector pGL3. The expression vector pGL3 comes from the Promega company and is registered under the Gene Accession Number U47296 at the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Bethesda, MD 20894, USA. The nucleotides 280 to 1932 were used as the luciferase gene. The sequence 26 nucleotides in length is a highly conserved sequence occurring in very many HCV genomes and their subtypes. In the HCV genome registered under the Gene Accession Number D89815 at the NCBI, the 26 nucleotides correspond to the nucleotides 9531 to 9556. They have the following sequence:

5'-gtcacggct agctgtgaa ggtccgt-3' (SEQ ID NO: 1).

The resulting fusion gene was cloned as a BamHI/NotI-DNA fragment in the eukaryotic expression plasmid pcDNA 3.1 (+) from the firm Invitrogen GmbH, Technologiepark Karlsruhe, Emmy-Noether Strasse 10, D-76131 Karlsruhe, catalog No. V790-20. The resulting plasmid is designated p8.

The plasmid pCMVßGal from the firm Clontech, Gene Accession Number U13186, NCBI, was used as a control for the transfection efficiency. This plasmid codes for the enzyme ß-galactosidase and brings about its expression.

The plasmid containing the fusion gene, the plasmid serving as the control, and various dsRNAs were introduced jointly by transfection into cells of the liver cell line HuH-7 (JCRB0403, Japanese Collection of Research Bioresources Cell Bank, National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan). The inhibition of the expression of the luciferase gene caused by the dsRNAs was determined in relation to the expression of the ß-galactosidase gene.

The dsRNAs used have the following sequences, designated in the sequence protocol as SEQ ID NO: 2 to SEQ ID NO: 13:

HCV1+2, whose strand S1 is totally complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5' - ACG GCU AGC UGU GAA AGG UCC GU-3' (SEQ ID NO: 2)
S1: 3' - AG UGC CGA UCG ACA CUU UCC AGG – 5' (SEQ ID NO:3)

HCV3+4, which is neither complementary to the HCV nor to the luciferase sequence in the fused HCV-luciferase gene and serves as a negative control:

S2: 5' - AGA CAG UCG ACU UCA GCC U GG-3' (SEQ ID NO: 12)
S1: 3'-GG UCU GUC AGC UGA AGU CGG A – 5' (SEQ ID NO: 13)

HCV5+6, whose strand S1, apart from the nucleotide printed in bold, is complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5' - ACG GCU AGC UGU GAA **U**GG UCC GU-3' (SEQ ID NO: 6)
S1: 3' - AG UGC CGA UCG ACA CUU **A**CC AGG – 5' (SEQ ID NO:7)

HCV7+8, whose strand S1, apart from the two nucleotides printed in bold, is complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5' - ACG GCA AGC UGU GAA UGG UCC GU-3' (SEQ ID NO: 8)
S1: 3' - AG UGC CGU UCG ACA CUU ACC AGG – 5' (SEQ ID NO:9)

Luc1+2, whose strand S1 is totally complementary to a luciferase sequence in the fused HCV-luciferase gene and which serves as the positive control:

S2: 5' - CGU UAU UUA UCG GAG UUG CAG UU-3' (SEQ ID NO: 10)
S1: 3' – GC GCA AUA AAU AGC CUC AAC GUC – 5' (SEQ ID NO: 11)

K3s+K3as, which is not complementary to either the HCV or a luciferase sequence in the fused HCV-luciferase gene and serves as the negative control:

S2: 5' – G AUG AGG AUC GUU UCG CAU GA-3' (SEQ ID NO: 4)
S1: 3'-UCC UAC UCC UAG CAA AGC GUA –5' (SEQ ID NO: 5)

HCV5+2, whose strand S1 is fully complementary to the HCV sequence and whose strand S2, apart from the nucleotide printed in bold, is complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5' - ACG GCU AGC UGU GAA UGG UCC GU-3' (SEQ ID NO: 6)
S1: 3' - AG UGC CGA UCG ACA CUU UCC AGG – 5' (SEQ ID NO:3)

HCV1+6, whose strand S2 is fully complementary to the HCV sequence and whose strand S1, apart from the nucleotide printed in bold, is complementary to the HCV sequence in the fused HCV-luciferase gene:

S2: 5' - ACG GCU AGC UGU GAA AGG UCC GU-3' (SEQ ID NO: 2)
S1: 3' - AG UGC CGA UCG ACA CUU ACC AGG – 5' (SEQ ID NO:7)

HuH-7 cells were cultured in DMEM with 10% FCS. To prepare for a transfection, 2 x 10E4 cells per well were seeded out in a 96-well cell culture plate. The cells were transfected 24 hours after being seeded by means of 110 µl of transfection medium per each well of the 96-well cell culture plate and continued to be cultured in this entire volume. Each transfection was carried out three times.

For this, at first 3 µg of the plasmid pCMVßGal were mixed with 1 µg of the plasmid p8. The transfection medium contained 0.25 µg of the mixture of plasmids and 200, 100, 50, 25, 12.5 or 0 nmol/l of one of the mentioned dsRNAs per well.

For the transfection, "Gene Porter 2" was used from the company PEQLAB Biotechnologie GmbH, Carl-Thiersch-Str. 2 b, D-91052 Erlangen, catalog number 13-T202007, according to the manufacturer's instructions.

The cells were then incubated at 37 degrees C and 5% CO2. One day after the transfection, 35 µl of fresh medium was added to each well and the cells were incubated for a further 24 h.

The effect of the dsRNAs used was determined by quantification of the expressed ß-galactosidase by means of "Galacto-Star" from the firm Tropix, 47 Wiggins Avenue, Bedford, MA 01730, USA, catalog number BM100S, and the effect of the expressed luciferase by means of "Luciferase" from the Tropix company, catalog number BC100L, through a chemiluminescence reaction. For this, lysates of the cells were prepared according to the manufacturer's instructions and 2 µl of this per assay was used for the detection of ß-galactosidase and 5 µl per assay for the detection of luciferase. The measurement of the chemiluminescence was done in a Sirius luminometer from Berthold Detection Systems GmbH, Bleichstrasse 56-68, D-75173 Pforzheim, Germany. The relative activity of the luciferase is determined as a measure of the expression, dividing the chemiluminescence value determined for luciferase by the value determined for ß-galactosidase. For every 3 values so determined, a mean is calculated. The mean for cells transfected

without dsRNA is arbitrarily defined as 1.0. The other means are placed in relation to this and shown by graph in Fig. 1 and 2.

Luc1+2 (positive control) resulted in the strongest inhibition of the luciferase activity (Fig. 1 and 2). In presence of HCV1+2, which was totally complementary to the target sequence for the reporter plasmid, a considerable reduction of the luciferase activity is likewise noticeable (Fig. 1 and 2). With decreasing concentration of HCV1+2, the luciferase activity increased. The oligonucleotide HCV5+6 not complementary to the target sequence in a nucleotide is similar to HCV1+2 in efficiency of luciferase inhibition, especially at low concentrations (Fig. 1). For the specificity of this dsRNA, this means that it is not enough for the dsRNA to be complementary to the target gene, yet it is not complementary to the original gene by one nucleotide, in order to inhibit the expression of the target gene specifically with respect to the expression of the original gene.

HCV7+8 inhibits the expression of luciferase both in high and also in low concentrations only to the degree of the negative controls HCV3+4 and K3S+K3AS (Fig. 1 and 2). The slight inhibition of the luciferase activity is to be deemed a nonspecific effect. For the specificity of this dsRNA, this means that it is enough for the dsRNA to be complementary to the target gene or not complementary by only one nucleotide, yet not complementary to the original gene in two nucleotides, in order to inhibit the expression of the target gene specifically with respect to the expression of the original gene.

In HCV5+2, one nucleotide in the sense strand S2 is not complementary to the antisense strand S1, while the antisense strand S1 is fully complementary to the target gene. This dsRNA is as efficient as LUC1+2 and HCV1+2 (Fig. 1 and 2). This is surprising, since a complementarity reduced by one base pair within the dsRNA would suggest a lower stability of the dsRNA in the cell and therefore a lower efficiency.

In HCV6+1, one nucleotide in the antisense strand S1 is not complementary to the sense strand S2, while the antisense strand S1 is also not complementary to the target gene by one nucleotide. HCV6+1 inhibits the expression less efficiently than HCV5+6, but more efficiently than HCV7+8 (Fig. 2). Thus, for the specificity and efficiency of the expression-inhibiting effect of the dsRNA, the sequence of the antisense strand S1 is more important than that of the sense strand S2.

HCV3+4 (Fig. 1) and K3S+K3AS (Fig. 2), serving as the negative control, resulted in little or no inhibition of the luciferase activity. The slight inhibition is nonspecific, since it does not depend on the concentration of the dsRNAs used.

The data show that at least two nucleotides not complementary to an original gene in the antisense strand of a dsRNA are needed to prevent an inhibition of the expression of the original gene. Furthermore, the data show that a modulation of the effectiveness of the inhibition of the expression by a dsRNA is possible by decreasing the degree of complementarity of the single strands forming the dsRNA.

Patent claims

1. Use of a double-stranded ribonucleic acid (dsRNA) for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein one strand S1 of the dsRNA has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene.

2. Use of a double-stranded ribonucleic acid (dsRNA) to make a medicament for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein one strand S1 of the dsRNA has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene.

3. Use per claim 1 or 2, wherein the nucleotide not complementary to the target gene is not situated at the 3' or 5' end of the region.

4. Use per one of the preceding claims, wherein the target gene has one or two point mutations with respect to the original gene.

5. Use per one of the preceding claims, wherein the original gene is a proto-oncogene and the target gene an oncogene derived from it.

6. Use per one of the preceding claims, wherein the cell is a tumor cell.

7. Use per one of the preceding claims, wherein one nucleotide of the region is not complementary to the target gene and two nucleotides of the region are not complementary to the original gene.

8. Use per one of the preceding claims, wherein at least one base pair is not complementary within the dsRNA.

9. Use per one of the preceding claims, wherein the dsRNA has a single-stranded projection formed from 1 to 4, especially 2 or 3 nucleotides at least at one end of the dsRNA.

10. Use per claim 9, wherein the dsRNA has the projection only at one end, especially its end having the 3' end of the strand S1.

11. Use per one of the preceding claims, wherein the dsRNA has, besides the strand S1, also a strand S2,and the strand S1 has a length of 23 nucleotides, the strand S2 a length of 21 nucleotides, and the 3' end of strand S1 a single-stranded projection formed from two nucleotides, while the end of the dsRNA located at the 5' end of the strand S1 is formed smooth in this case.

12. Use per one of the preceding claims, wherein the strand S1 is complementary to the primary or processed RNA transcript of the target gene.

13. Use per one of the preceding claims, wherein the dsRNA is present in a preparation that is suitable for inhalation, oral ingestion, infusion and injection, especially for intravenous, intraperitoneal or intratumoral infusion or injection.

14. Use per claim 13, wherein the preparation consists, and especially consists solely of a physiologically tolerated solvent, preferably a physiological salt solution or a physiologically tolerated buffer, especially a phosphate-buffered salt solution, and the dsRNA.

15. Use per one of the preceding claims, wherein the dsRNA is present in a physiologically tolerated solution, especially a physiologically tolerated buffer or a physiological salt solution, or enveloped by a micelle structure, preferably a liposome, a virus capsid, a capsoid or a polymer nanocapsule or microcapsule, or bound to a polymer nanocapsule or microcapsule.

16. Use per one of the preceding claims, wherein the dsRNA is administered orally, by means of inhalation, infusion or injection, especially intravenous, intraperitoneal or intratumoral infusion or injection.

17. Use per one of the preceding claims, wherein the dsRNA is used in a dosage of not more than 5 mg, especially not more than 2.5 mg, preferably not more than 200 µg, especially preferably not more than 100 µg, preferably not more than 50 µg, especially not more than 25 µg, per kg of body weight and per day.

18. Medicament for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein the medicament contains a double-stranded ribonucleic acid (dsRNA), whose one strand S1 has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene.

19. Medicament per claim 18, wherein the nucleotide not complementary to the target gene is not situated at the 3' or 5' end of the region.

20. Medicament per claims 18 or 19 wherein the target gene has one or two point mutations with respect to the original gene.

21. Medicament per one of claims 18 to 20, wherein the original gene is a proto-oncogene and the target gene an oncogene derived from it.

22. Medicament per one of claims 18 to 21, wherein the cell is a tumor cell.

23. Medicament per one of claims 18 to 22, wherein one nucleotide of the region is not complementary to the target gene and two nucleotides of the region are not complementary to the original gene.

24. Medicament per one of claims 18 to 23, wherein at least one base pair is not complementary within the dsRNA.

25. Medicament per one of claims 18 to 24, wherein the dsRNA has a single-stranded projection formed from 1 to 4, especially 2 or 3 nucleotides at least at one end of the dsRNA.

26. Medicament per claim 25, wherein the dsRNA has the projection only at one end, especially its end having the 3' end of the strand S1.

27. Medicament per claim 26, wherein the dsRNA has, besides the strand S1, also a strand S2,and the strand S1 has a length of 23 nucleotides, the strand S2 a length of 21 nucleotides, and the 3' end of strand S1 a single-stranded projection formed from two nucleotides, while the end of the dsRNA located at the 5' end of the strand S1 is formed smooth in this case.

28. Medicament per one of claims 18 to 27, wherein the strand S1 is complementary to the primary or processed RNA transcript of the target gene.

29. Medicament per one of claims 18 to 28, wherein the dsRNA is present in a preparation that is suitable for inhalation, oral ingestion, infusion and injection, especially for intravenous, intraperitoneal or intratumoral infusion or injection.

30. Medicament per claim 29, wherein the preparation consists, and especially consists solely of a physiologically tolerated solvent, preferably a physiological salt solution or a physiologically tolerated buffer, especially a phosphate-buffered salt solution, and the dsRNA.

31. Medicament per one of claims 18 to 30, wherein the dsRNA in the medicament is present in a solution, especially a physiologically tolerated buffer or a physiological salt solution, or enveloped by a micelle structure, preferably a liposome, a virus capsid, a capsoid or a polymer nanocapsule or microcapsule, or bound to a polymer nanocapsule or microcapsule.

32. Medicament per one of claims 18 to 31, wherein the medicament is present in at least one administration unit which contains the dsRNA in an amount that enables a dosage of not more than 5 mg, especially not more than 2.5 mg, preferably not more than 200 µg, especially preferably not more than 100 µg, preferably not more than 50 µg, especially not more than 25 µg, per kg of body weight and per day.

33. Method for specifically inhibiting the expression of a given target gene, having a point mutation with respect to an original gene, in a cell, wherein a double-stranded ribonucleic acid (dsRNA) is introduced into the cell and one strand S1 of the dsRNA has a region complementary to the target gene, in which at least one nucleotide is not complementary to the target gene, and the number of nucleotides which are not complementary to the original gene is at least one more than the number of nucleotides which are not complementary to the target gene.

34. Method per claim 33, wherein the nucleotide not complementary to the target gene is not situated at the 3' or 5' end of the region.

35. Method per claim 33 or 34, wherein the target gene has one or two point mutations with respect to the original gene.

36. Method per one of claims 33 to 35, wherein the original gene is a proto-oncogene and the target gene an oncogene derived from it.

37. Method per one of claims 33 to 36, wherein the cell is a tumor cell.

38. Method per one of claims 33 to 37, wherein one nucleotide of the region is not complementary to the target gene and two nucleotides of the region are not complementary to the original gene.

39. Method per one of claims 33 to 38, wherein at least one base pair is not complementary within the dsRNA.

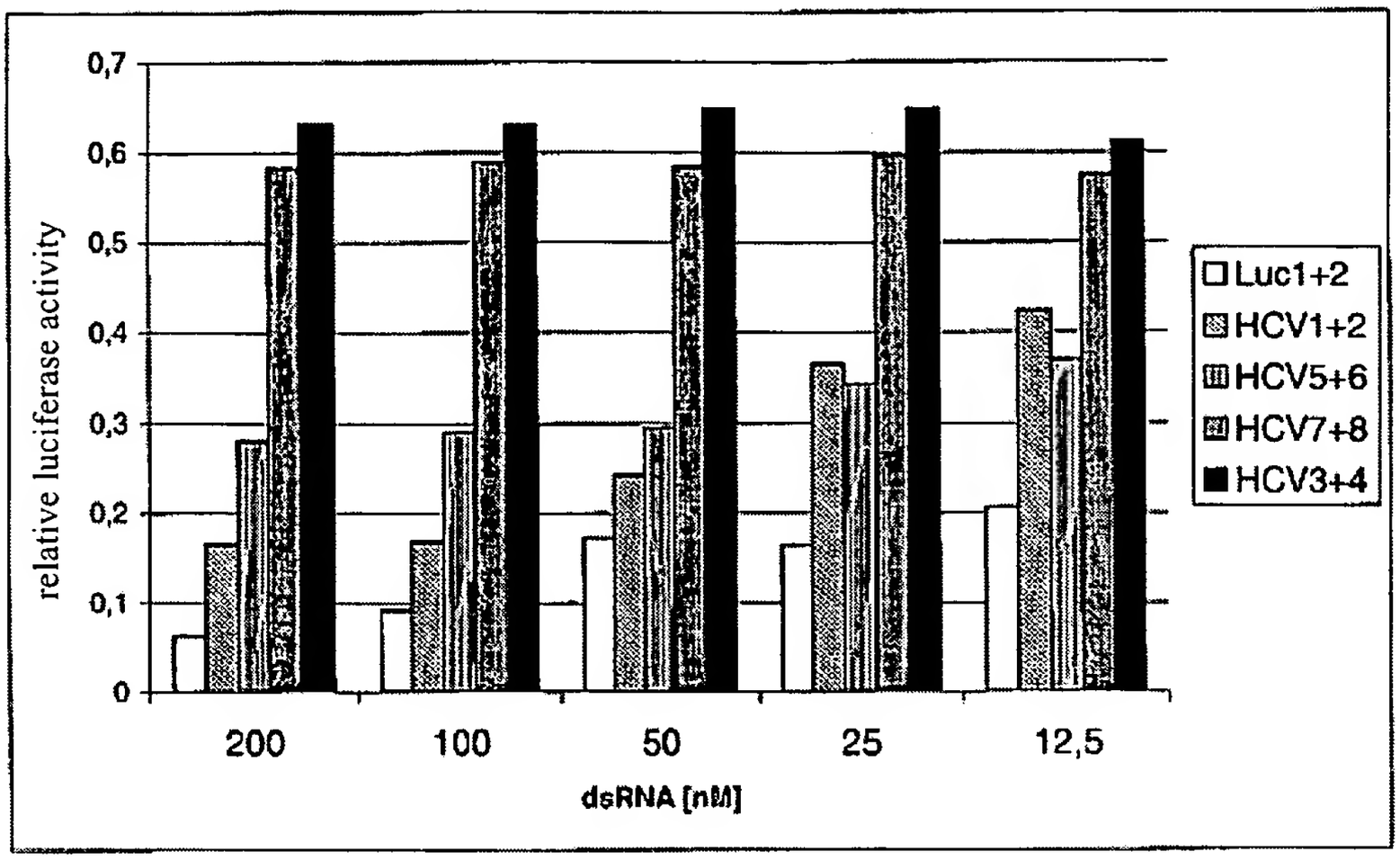
40. Method per one of claims 33 to 39, wherein the dsRNA has a single-stranded projection formed from 1 to 4, especially 2 or 3 nucleotides at least at one end of the dsRNA.

41. Method per claim 405, wherein the dsRNA has the projection only at one end, especially its end having the 3' end of the strand S1.

42. Method per claim 41, wherein the dsRNA has, besides the strand S1, also a strand S2,and the strand S1 has a length of 23 nucleotides, the strand S2 a length of 21 nucleotides, and the 3' end of strand S1 a single-stranded projection formed from two nucleotides, while the end of the dsRNA located at the 5' end of the strand S1 is formed smooth.

43. Method per one of claims 33 to 42, wherein the strand S1 is complementary to the primary or processed RNA transcript of the target gene.

44. Method per one of claims 33 to 43, wherein the dsRNA is present in a solution, especially a physiologically tolerated buffer or a physiological salt solution, or enveloped by a micelle structure, preferably a liposome, a virus capsid, a capsoid or a polymer nanocapsule or microcapsule, or bound to a polymer nanocapsule or microcapsule.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HCV1+2
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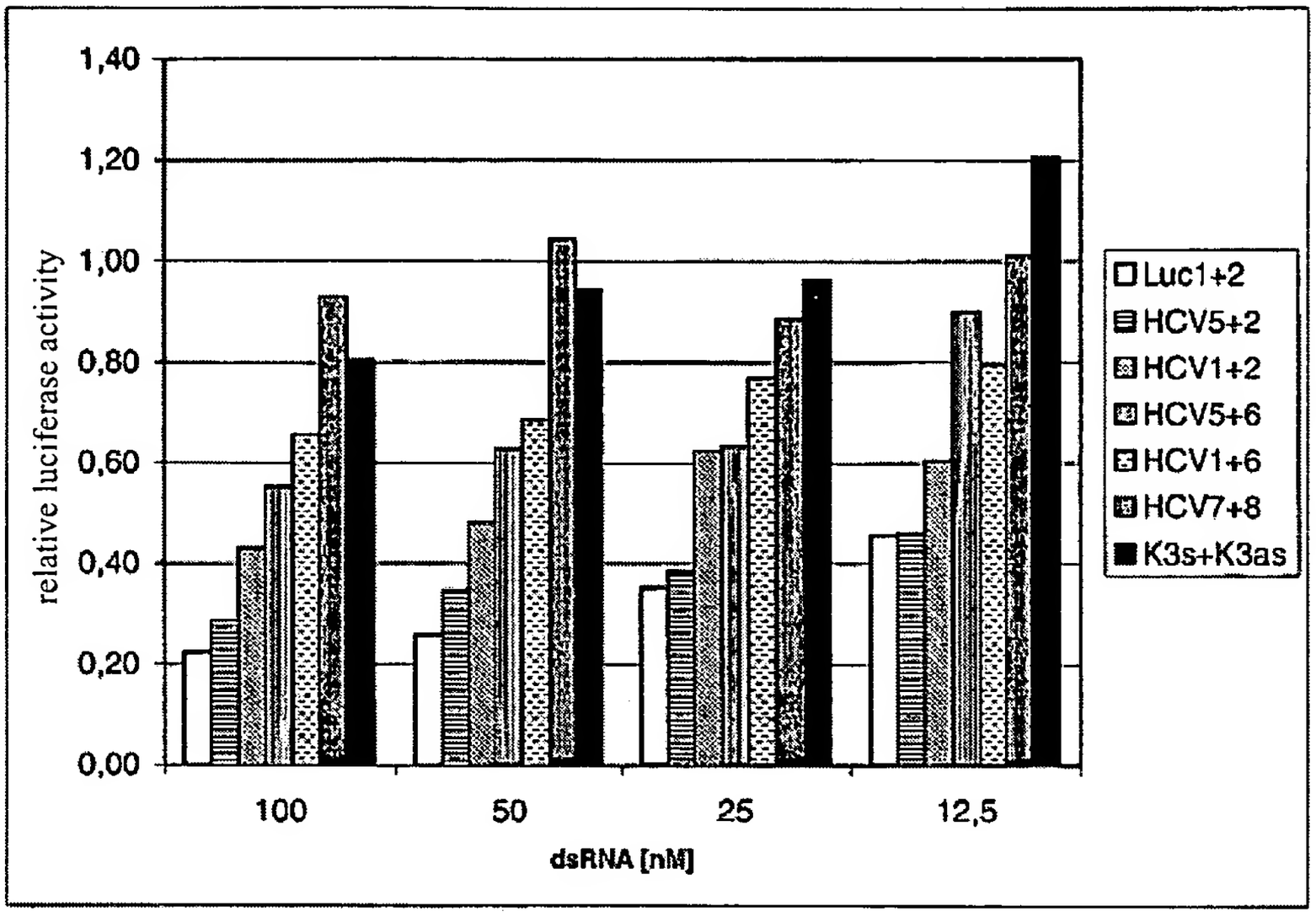
HCV3+4

Fig. 1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dsRNA [nM]
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HCV5+2
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Fig. 2

SEQUENCE PROTOCOL

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<120> Use of a double-stranded ribonucleic acid for specifically inhibiting the expression of a given target gene

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